REMARKS

The Office is authorized to charge the fee for the extension of time to Deposit Account No. 02-1818. Any fees that may be due in connection with the filing of this paper or with this application may be charged to Deposit Account No. 02-1818. If a Petition for Extension of Time is needed, this paper is to be considered such Petition.

A Supplemental Information Disclosure Statement and a Form PTO-1449 is provided under separate cover.

Claims 1-7, 9, 11-16, 45, 48, 50-54, 56-59, 61-63 and 65-78 are pending. Claims 1, 53, 59 and 63 are amended for clarity and to correct typographical errors, and claims 50 and 54 are cancelled. No new matter is added.

Information Disclosure Statement

In the Office Action dated January 20, 2010, the Examiner alleges that the Information Disclosure Statement filed on October 1, 2009, fails to comply with provisions 37 C.F.R. §§1.97, 1.98 and MPEP §609 because "the list of other information i.e., copy of examination report from Australia and New Zealand submitted for consideration is not listed in a section separately from citations of other documents." Applicant respectfully disagrees. The Supplemental Information Disclosure Statement (sIDS) filed on October 1, 2009, included a table (i.e., a separate section) listing the Australian and New Zealand Examination Reports. In compliance with 37 CFR §1.98 (a)(1), (a)(2) and (a)(3), the sIDS also included the application number of the application in which the sIDS was being submitted, a column that provided a blank space next to each citation for the Examiner's initials, a heading on the listing that clearly indicated the list is an Information Disclosure Statement, and copies of Documents 1 and 2. Therefore, the sIDS filed on October 1, 2009, is in compliance with 37 CFR §§ 1.97, 1.98 and MPEP § 609, and Applicant respectfully requests that Examiner consider and initial all information listed in the table.

The Examiner is requested to please review the file history of this application in PAIR. When submitting Information Disclosure Statements, the undersigned includes a transmittal letter, a paper providing information (IDS letter), and a Form PTO-1449. In some instances, the IDS letter includes a table (i.e., a separate section) with documents that require initials by the Examiner. It has come to the attention of the undersigned that Information Disclosure Statements have been misclassified in PAIR. Also the documents have been separated such that, at best, only parts of the documents are getting to the Examiner for review. In the Supplemental Information Disclosure Statements filed on August 22, 2007;

September 12, 2008; June 11, 2009; July 10, 2009; October 1, 2009; and March 31, 2010, the transmittal letter and accompanying IDS letter providing information have been classified together as a transmittal letter (TRAN.LET). To address this, the undersigned contacted the Examiner on March 10, 2010, and requested that documents submitted in the Information Disclosure Statement tables be considered and initialed by the Examiner. The Examiner agreed to consider the documents submitted in the Information Disclosure Statement tables, providing a statement is included herewith in the response to the Office Action dated January 20, 2010.

I. THE REJECTION OF CLAIMS 1-7, 9, 11-16, 45, 48, 50-54, 56-59, 61-63 AND 65-78 UNDER 35 U.S.C. §112, FIRST PARAGRAPH

Claims 1-7, 9, 11-16, 45, 48, 50-54, 56-59, 61-63 and 65-78 are rejected under 35 U.S.C. §112, first paragraph, for alleged lack of written description, because it is alleged that the specification does not describe the subject matter in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s) had possession of the claimed subject matter at the time the specification was filed. Specifically, the Examiner alleges that the amendments to claims 1, filed with the Election and Amendment on June 11, 2009, introduces new matter. Further, the Examiner urges that the disclosure does not describe muteins that inactivate an activity of a target protein, or the genus of proteases employed in the methods. These rejections respectfully are traversed.

Relevant Law

The purpose behind the written description requirement is to ensure that the patent applicant had possession of the claimed subject matter at the time of filing of the application In re Wertheim, 541 F.2d 257, 262, 191 USPQ 90, 96 (CCPA 1976). The manner in which the specification meets the requirement is not material; it may be met by either an express or an implicit disclosure.

35 U.S.C. §112 requires a written description of the invention. This requirement is distinct from and not coterminous with the enablement requirement:

> The purpose of the 'written description' requirement is broader than to merely explain how to 'make and use'; the applicant must also convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention. The invention is, for purposes of the 'written description' inquiry, whatever is now claimed." *Vas-Cath, Inc. v. Mahurkar*, 935 F.2d at 1563-64, 19 USPQ2d at 1117 (emphasis in original).

The issue with respect to 35 U.S.C. §112, first paragraph, adequate written description has been stated as:

> [d]oes the specification convey clearly to those skilled in the art, to whom it is addressed, in any way, the information that appellants invented that specific compound [claimed embodiment] *Vas-Cath, Inc. v. Mahurkar*, at 1115, quoting *In re Ruschig*, 390 F.2d 1990, at 995-996, 154 USPQ 118 at 123 (CCPA 1967).

A specification must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention, *i.e.,* whatever is now claimed. *Vas-Cath, Inc. v. Mahurkar*, 935 F.2d 1555, 1563-64, 19 USPQ.2d 1111, 1117 (Fed. Cir. 1991). A written description requirement issue generally involves the question of whether the subject matter of a claim is supported by or conforms to the disclosure of an application as filed. The test for sufficiency of support in a patent application is whether the disclosure of the application relied upon "reasonably conveys to the artisan that the inventor had possession at that time of the later claimed subject matter." *Ralston Purina Co. v. Far-Mar-Co., Inc.,* 772 F.2d 1570, 1575, 227 USPQ 177, 179 (Fed. Cir. 1985) (quoting In re Kaslow, 707 F.2d 1366, 1375, 217 USPQ 1089, 1096 (Fed. Cir. 1983)) (see also, MPEP 2163.02).

An objective standard for determining compliance with the written description requirement is "does the description clearly allow persons of skill in the art to recognize that he or she invented what is claimed." In re Gosteli, 872 F.2d 1008, 1012, 10 USPQ.2d 1614, 1618 (Fed. Cir. 1989).

The Examiner has the initial burden of presenting evidence or reasons why persons skilled in the art would not recognize in an applicant's disclosure a description of the invention defined by the claims. In re Wertheim, 541 F.2d 257, 265, 191 USPQ 90, 98 (CCPA 1976); *See also* Ex parte Sorenson, 3 USPQ.2d 1462, 1463 (Bd. Pat.App. & Inter. 1987). By disclosing in a parent application a device that inherently performs a function or has a property, operates according to a theory or has an advantage, a patent application necessarily discloses that function, theory or advantage, even though it says nothing explicit concerning it. The application may later be amended to recite the function, theory or advantage without introducing prohibited new matter. In re Reynolds, 443, F.2d 384, 170 USPQ 94 (CCPA 1971); and In re Smythe, 480 F.2d 1376, 178 USPQ 279 (CCPA 1973).

Furthermore, the subject matter of the claims need not be described literally (i.e. using the same terms or inhaec verba) in order for the disclosure to satisfy the description requirement. If a claim is amended to include subject matter, limitations, or terminology not present in the application as filed, involving a departure from, addition to, or deletion from

the disclosure of the application as filed, the examiner should conclude that the claimed subject matter is not described in that application. This conclusion will result in the rejection of the claims affected under 35 U.S.C. 112, first paragraph – description requirement, or denial of the benefit of the filing date of a previously filed application, as appropriate.

The claims

The claims are directed to **methods** of identifying a mutein protease that cleaves a substrate sequence in a target protein involved with a pathology by identifying those protease mutants from among a library of mutants that have increased cleavage activity and/or altered specificity for a substrate sequence in the target substrate. Cleavage of the target protein by the mutein protease inactivates an activity of the target protein. By virtue of the methods, the protease muteins that inactivate a target protein involved in a disease or pathology are identified. There is no *a priori* requirement to have any knowledge of the structure or function, including specificity and activity, of a starting protease before performing the method. The purpose of the method is to identify proteases (or protease domains thereof), by screening a collection and identifying those that cleave a sequence in a target protein. By following the method, **mutant proteases that cleave the target protein with specificity and activity** are identified. For example:

Independent claim 1 recites:

> A method of producing and identifying a mammalian protease mutein that inactivates an activity of a target protein involved with a disease or pathology in a mammal, wherein:
>
> the target protein is selected from among a cell surface molecule that transmits an extracellular signal for cell proliferation, a cytokine, a cytokine receptor and a signaling protein that regulates apoptosis;
>
> inactivation of the target protein can ameliorate a disease or pathology;
>
> cleavage of a substrate sequence in the target protein inactivates an activity of the target protein; and
>
> the method comprises the steps of:
>
> (a) producing a library of protease muteins of a protease scaffold and/or biologically active portions thereof, wherein:
>
> each different mutein protease in the library is a member of the library;
>
> each member of the library has N mutations relative to a wild-type mammalian protease scaffold or a biologically active portion thereof; and
>
> N is a positive integer;
>
> (b) contacting members of the library with the target protein or with a polypeptide comprising a substrate sequence that is present in the target protein;
>
> (c) measuring a cleavage activity and/or substrate specificity of at least two members of the library for the target protein or substrate sequence;
>
> (d) based on the measured activity and/or specificity, identifying members of the library that have an increased cleavage activity and/or altered substrate specificity for

cleaving the substrate sequence or the target protein, relative to the wild-type mammalian protease scaffold; and

(e) testing the identified protease(s) for cleavage and inactivation of an activity of the target protein that contains the substrate sequence, thereby identifying a protease mutein or a biologically active portion thereof that inactivates an activity of a target protein that is involved with the disease or pathology.

Dependent claims specify particulars of the method. For example, dependent claims 3-6 specify the N number of mutations in each member of the library of mutant proteases. Dependent claim 2 specifies that the protease is a cysteine or serine protease and dependent claim 7 specifically recites the starting protease scaffold used in the method. Further independent claims recite as follows:

Independent claim 53 recites:

A method of producing and identifying a mammalian protease mutein that inactivates an activity of a target protein involved with a disease or pathology in a mammal, wherein:

inactivation of the target protein can ameliorate a disease or pathology;

cleavage of a substrate sequence in the target protein inactivates an activity of the target protein;

the mammalian protease is selected from among a granzyme A, granzyme B, granzyme M, cathepsin, trypsin, chymotrypsin, subtilisin, MTSP-1, elastase, chymase, tryptase, collagenase, papain, neutrophil elastase, complement factor serine proteases, ADAMTS13, neural endopeptidase/neprilysin, furin, cruzain, and urokinase plasminogen activator (uPA); and

the method comprises the steps of:

(a) producing a library of protease muteins of a protease scaffold and/or biologically active portion thereof, wherein:

each different protease mutein in the library being a member of the library;

each member of the library has N mutations relative to a wild-type mammalian protease scaffold or a biologically active portion thereof; and

N is a positive integer;

(b) contacting members of the library with the target protein or with a polypeptide comprising a substrate sequence that is present in the target protein;

(c) measuring a cleavage activity and/or substrate specificity of at least two members of the library for the target protein or substrate sequence;

(d) based on the measured activity and/or specificity, identifying those members of the library that have an increased cleavage activity and/or altered substrate specificity for cleaving the substrate sequence or the target protein relative to the wild-type mammalian protease scaffold;

(e) testing the identified mutein protease(s) or biologically active portion thereof for cleavage and inactivation of an activity of the target protein that contains the substrate sequence, thereby identifying a protease mutein or a biologically active portion thereof that inactivates an activity of a target protein that is involved with or causes the disease or pathology

(f) identifying the mutation(s) contained in a first mutein protease and a second mutein protease identified in step (d) and step (e) as having increased cleavage activity and/or altered specificity for cleaving the target protein or substrate sequence and that can inactivate an activity of the target protein;

(g) generating a third mutein protease containing the mutations of the first mutein protease and the mutations of the second mutein;

(h) measuring the cleavage activity and/or substrate specificity of the third mutein protease to determine whether the third mutein protease has increased cleavage activity and/or altered specificity toward the target protein or substrate sequence compared to the first mutein protease or second mutein protease; and

(i) testing the third mutein for cleavage and inactivation of an activity of the target protein that contains the substrate sequence, thereby identifying a protease mutein or a biologically active portion thereof that inactivates an activity of a target protein that is involved with or causes the disease or pathology.

Independent claim 59 recites:

A method of producing and identifying a human protease mutein that inactivates an activity of a target protein involved with a disease or pathology in a human, wherein:

cleavage of a substrate sequence in the target protein inactivates an activity of the target protein;

the target protein is selected from among a cell surface molecule that transmits an extracellular signal for cell proliferation, a cytokine, a cytokine receptor and a signaling protein that regulates apoptosis;

inactivation of the target protein can ameliorate a disease or pathology; and

the method comprises the steps of:

(a) producing a library of protease muteins of a protease scaffold and/or biologically active portions thereof, wherein:

each different protease mutein in the library is a member of the library;

each member having N mutations relative to a wild-type human protease scaffold or a biologically active portion thereof, wherein N is a positive integer; and

the human protease scaffold is selected from among a granzyme A, granzyme B, granzyme M, cathepsin, trypsin, chymotrypsin, subtilisin, MTSP-1, elastase, chymase, tryptase, collagenase, papain, neutrophil elastase, complement factor serine proteases, ADAMTS13, neural endopeptidase/neprilysin, furin, cruzain, and urokinase plasminogen activator (uPA);

(b) contacting members of the library with the target protein or with a polypeptide comprising a substrate sequence that is present in the target protein;

(c) measuring a cleavage activity and/or substrate specificity of at least two members of the library for the substrate sequence or the target protein; and

(d) based on the measured activity and/or specificity, identifying those members of the library that have an increased cleavage activity and/or altered substrate specificity for cleaving the substrate sequence or the target protein relative to the wild-type human protease scaffold,; and

(e) testing the identified protease(s) for cleavage and inactivation of an activity of the target protein that contains the substrate sequence, thereby identifying a protease mutein or a biologically active portion thereof that inactivates an activity of a target protein that is involved with or causes the disease or pathology.

Independent claim 63 recites:

A method of producing and identifying a human protease mutein that inactivates an activity of a target protein involved with a disease or pathology in a mammal, wherein:

cleavage of a substrate sequence in the target protein inactivates an activity of the target protein;

the target protein is selected from among a caspase 3, tumor necrosis factor, tumor necrosis factor receptor, interleukin-1, interleukin-1 receptor, interleukin-2, interleukin-2 receptor, interleukin-4, interleukin-4 receptor, interleukin-5, interleukin-5 receptor, interleukin-12, interleukin-12 receptor, interleukin-13, interleukin-13 receptor, p-selectin, p-selectin glycoprotein ligand, Substance P, Bradykinin, PSGL, factor IX, immunoglobulin E, immunoglobulin E receptor, CCR5, CXCR4, glycoprotein 120, glycoprotein 41, hemagglutinin, respiratory syncytium virus fusion protein, B7, CD28, CD2, CD3, CD4, CD40, vascular endothelial growth factor, VEGF receptor, fibroblast growth factor, endothelial growth factor, EGF receptor, TGF receptor, transforming growth factor, Her2, CCR1, CXCR3, CCR2, Src, Akt, Bcl-2, BCR-Abl, glucagon synthase kinase-3, cyclin dependent kinase-2 (cdk-2), and cyclin dependent kinase-4 (cdk-4);

inactivation of the target protein can ameliorate a disease or pathology; and

the method comprises the steps of:

(a) producing a library of human protease muteins of a protease scaffold and/or biologically active portions thereof, wherein:

each different protease mutein in the library is a member of the library;

each member having N mutations relative to a wild-type human protease scaffold or a biologically active portion thereof, wherein N is a positive integer; and

the human protease scaffold is selected from among a granzyme A, granzyme B, granzyme M, cathepsin, MTSP-1, elastase, chymase, tryptase, chymotrypsin, collagenase, factor Xa, Protein C, plasma kallikrein, plasmin, trypsin, thrombin, complement factor serine proteases, papain, ADAMTS13, endopeptidase, furin, cruzain and urokinase plasminogen activator (uPA); and

(b) contacting members of the library with the target protein or with a polypeptide comprising a substrate sequence that is present in the target protein;

(c) measuring a cleavage activity and/or substrate specificity of at least two members of the library for the substrate sequence or the target protein; and

(d) based on the measured activity and/or specificity, identifying those members of the library that have an increased cleavage activity and/or altered substrate specificity for cleaving the substrate sequence or the target protein relative to the wild-type human protease scaffold; and

(e) testing the identified protease(s) for cleavage and inactivation of an activity of the target protein that contains the substrate sequence, thereby identifying a protease mutein or a biologically active portion thereof that inactivates an activity of a target protein that is involved with the disease or pathology.

Each of independent claims 53, 59 and 63 recite the specific protease scaffolds used in the method. The dependent claims recite specifics of the methods.

Analysis

1. The rejection of claim 1 as allegedly containing new matter

Claim 1 is rejected under 35 U.S.C. §112, first paragraph, for allegedly containing new matter. The Examiner asserts that "Claim 1 which recites "inactivation can ameliorate a disease or pathology" is not supported in the as-filed specification." Further, the Examiner states that:

> It is not readily apparent from the given paragraphs which amendments find support therein. The claim to "a **polypeptide** comprising a substrate sequence..." is also not in the original disclosure. The original disclosure cited by applicants at *e.g.*, paragraphs [0050] and [0125] do not recite a polypeptide rather the protein per se with the substrate sequence. Thus, it is not apparent as to the residues, length or location of the polypeptide relative to the protein. Furthermore, the support for the protease scaffold "and catalytically active portions thereof" is not provided in any of the paragraph sections cited by applicants for support.

The Examiner requests that applicant identifies the passages in the application from which the amendments finds basis. Provided below is a detailed description of where in the specification as filed the claim amendments find basis.

In the Preliminary Amendment and Request for Continued Examination filed on June 11, 2009, claim 1 was amended as follows (the amendments discussed below are identified as A-J):

> A method of producing and identifying a mammalian protease mutein **(A)** that inactivates an activity of ~~with increased cleavage activity and/or altered substrate specificity for~~ a target protein involved with a **(B)**disease or pathology in a mammal, wherein:
>
> the target protein is selected from among a cell surface molecule that transmits an extracellular signal for cell proliferation, a cytokine, a cytokine receptor and a signaling protein that regulates apoptosis **(C)**whose inactivation can ameliorate a disease or pathology;
>
> cleavage of a substrate sequence in [[said]] the target protein **(D)** inactivates an activity of the target protein ~~serves as a treatment for said pathology~~; and
>
> the method comprises the steps of:
>
> (a) producing a library of protease muteins of a protease scaffold **(E)**and/or catalytically active portions thereof, wherein:
>
> each different mutein protease in the library is a member of the library;
>
> each member of the library has N mutations relative to a wild-type mammalian protease scaffold **(F)**or a catalytically active portion thereof; and
>
> N is a positive integer;
>
> (b) **(G)**contacting members of the library with the target protein or with a polypeptide comprising a substrate sequence that is present in the target protein;
>
> ~~(b)~~ (c) measuring a cleavage activity and/or substrate specificity of at least two members of the library for the **(H)**target protein or substrate sequence; and

~~(c)~~ (d) (I)based on the measured activity and/or specificity, identifying members of the library that have ~~at least one mutein protease having~~ an increased cleavage activity and/or altered substrate specificity for cleaving ~~said~~ the substrate sequence ~~in~~ or the target protein, relative to the wild-type mammalian protease scaffold, (J) thereby identifying a protease mutein or a catalytically active portion thereof that inactivates an activity of a target protein that is involved with the disease or pathology, whereby the identified protease is a candidate therapeutic for treatment of the disease or pathology.

Provided below are the passages in the application that form the basis of the amendments identified as A-J.

A. Claim 1 is amended to recite that the mammalian protease mutein inactivates an activity of a target protein. Basis for this amendment can be found, for example, on page 9, lines 10-21, which states:

> The present invention is drawn to methods for generating and screening proteases to cleave target proteins at a given substrate sequence. Proteases are protein-degrading enzymes that recognize an amino acid or an amino acid substrate sequence within a target protein. Upon recognition of the substrate sequence, proteases catalyze the hydrolysis or cleavage of a peptide bond within a target protein. **Such hydrolysis of the target protein may inactivate it**, depending on the location of peptide bond within the context of the full-length sequence of the target sequence. The specificity of proteases can be altered through protein engineering. If a protease is engineered to recognize a substrate sequence within a target protein or proteins that would *(i.)* **alter the function *i.e.* by inactivation of the target protein(s)** upon catalysis of peptide bond hydrolysis and, *(ii.)* the target protein(s) are recognized or unrecognized as points of molecular intervention for a particular disease or diseases, then the engineered protease has a therapeutic effect via a proteolysis-mediated inactivation event. [emphasis added]

Further basis can be found, for example, on page 11, lines 1-3, which states:

> In some examples, the engineered protease is designed to cleave any of the target proteins in Table 1, **thereby inactivating the activity of the protein**. The protease can be used to treat a pathology associated with that protein, by inactivating one of the target proteins. [emphasis added]

B. Claim 1 is amended to recite that the target protein is involved in a disease. Basis for this amendment can be found, for example, on page 2, lines 21-22, which states:

> The present invention is drawn to the generation and screening of proteases that cleave proteins known to be involved in disease.

Further basis can be found, for example, on page 9, lines 17-20, which states:

> If a protease is engineered to recognize a substrate sequence within a target protein or proteins that would *(i.)* alter the function *i.e.* by inactivation of the target protein(s) upon catalysis of peptide bond hydrolysis and, *(ii.)* **the target protein(s) are recognized or unrecognized as points of molecular intervention for a particular disease or diseases,** then the engineered protease has a therapeutic effect via a proteolysis-mediated inactivation event. [emphasis added]

C. Claim 1 is amended to recite that inactivation of the target protein can ameliorate a disease or pathology. It respectfully is submitted that the term "ameliorate" is used in the specification in a similar manner to, and interchangeably with, the term

"treat." This is demonstrated, for example, on page 38, lines 25-31, which describes the use of a therapeutically effective amount of a protease inhibitor to treat or prevent, **i.e. ameliorate**, an apoptosis-associated disorder:

> In one aspect, the invention provides a method of **treating or preventing** an apoptosis-associated disorder in a subject in need thereof by administering to the subject a therapeutically effective amount of a protease-inhibitor so apoptosis is inhibited. The subject can be *e.g.*, any mammal, *e.g.*, a human, a primate (*e.g.*human), mouse, rat, dog, cat, cow, horse, or pig. The term "therapeutically effective" means that the amount of protease-inhibitor, for example, which is used, is of sufficient quantity to **ameliorate** the apoptosis-associated disorder [emphasis added]

The application describes in multiple passages the fact that the cleavage and inactivation of the target protein serve to treat (i.e. ameliorate) the disease or pathology associated with the target protein. For example, on page 9, lines 26-32, states:

> In one embodiment, the target protein to be cleaved is involved with a pathology, where cleaving the target protein at a given substrate sequence serves as a treatment for the pathology.
>
> In one embodiment, the protease cleaves a protein involved with rheumatoid arthritis. For example, the protease cleaves the TNF receptor between the transmembrane domain and the cytokine binding domain. This cleavage can inactivate the receptor. Rheumatoid arthritis is thereby treated by inhibiting the action of tumor necrosis factor (TNF).

On page 10, lines 22-26, which states:

> In another embodiment of the invention, the protease cleaves cytokines or receptors that are involved in inflammation as a treatment for asthma or other pathologies associated with inflammation. By cleaving the cytokine or receptors, the protease can inactivate the signaling cascade involved with many inflammatory processes. The protease can thereby be used to treat inflammation and related pathologies.

Thus, it respectfully is submitted that recitation in claim 1 that inactivation of the target protein can ameliorate a disease or pathology finds basis in the application as filed.

D. Claim 1 is amended to recite that cleavage of the target protein inactivates an activity of a target protein. Basis for this amendment can be found, for example, on page 11, lines 1-3, which states:

> In some examples, the engineered protease is designed to **cleave** any of the target proteins in Table 1, **thereby inactivating the activity of the protein**. The protease can be used to treat a pathology associated with that protein, by inactivating one of the target proteins. [emphasis added]

E and F. Claim 1 is amended to include reference to catalytically active portions of a protease. The Examiner's attention is directed to the instant amendment of claims to recite a biologically active portion. Basis for this amendment can be found, for example, on page 34, line 18 through page 35, line 3, which describes biologically-active portions of the proteases:

> One aspect of the invention pertains to isolated proteases, and biologically active portions thereof, or derivatives, fragments, analogs or homologs thereof. Also provided

> are polypeptide fragments suitable for use as immunogens to raise anti protease antibodies. In another embodiment, proteases are produced by recombinant DNA techniques. Alternative to recombinant expression, a protease protein or polypeptide can be synthesized chemically using standard peptide synthesis techniques.
>
> Biologically active portions of protease proteins include peptides comprising amino acid sequences sufficiently homologous to or derived from the amino acid sequences of the protease proteins that include fewer amino acids than the full length protease proteins, and exhibit at least one activity of a protease protein. Typically, biologically active portions comprise a domain or motif with at least one activity of the protease protein. A biologically active portion of a protease protein is a polypeptide which is, for example, 10, 25, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300 or more amino acid residues in length.
>
> Moreover, other biologically active portions of a protein, in which other regions of the protein are deleted, can be prepared by recombinant techniques and evaluated for one or more of the functional activities of a native protease.

G. Claim 1 is amended to recite the step of contacting members of the library with the target protein or with a polypeptide comprising a substrate sequence that is present in the target protein. Basis for this amendment can be found, for example, on page 2, line 29 through page 3, line 4, which states:

> One embodiment of the invention involves generating a library of protease sequences to be used to screen for modified proteases that cleave a desired target protein at a desired substrate sequence. In one aspect of this embodiment, each member of the library is a protease scaffold with a number of mutations made to each member of the library. A protease scaffold has the same or a similar sequence to a known protease. In one embodiment, this scaffold is a serine protease. In another embodiment of the invention, this scaffold is a cysteine protease. The cleavage activity of each member of the library is measured using the desired substrate sequence from the desired target protein.

Further basis can be found, for example, on page 17, lines 18-21, which states:

> Libraries are screened to ascertain the substrate sequence specificity of the members. Libraries of scaffolds are tested for specificity by exposing the members to substrate peptide sequences.

Pages 28 through 32 describe the various assays that utilize either the full length target protein (see *e.g.* page 32, lines 22-23) or a peptide (a term that can be used interchangeably with "polypeptide") that contains the substrate sequence (see *e.g.* page 29, lines 12-14) to assay for the specificity of the muteins.

H. Claim 1 is amended to recite that the method includes measuring cleavage activity and/or substrate specificity for the target protein. Basis for this amendment can be found, for example, on page 3, lines 3-4, which states:

> The cleavage activity of each member of the library is measured using the desired substrate sequence from the desired target protein.

Further basis can be found, for example, on page 32, lines 22-25, which states:

> Variant proteases are also assayed to ascertain that they will cleave the desired sequence when presented in the context of the full-length protein. The activity of the target protein is also assayed to verify that its function has been destroyed by the cleavage event.

I. Claim 1 is amended to recite that the method identifies a protease mutein or a catalytically active portion thereof that inactivates an activity of a target protein that is involved with the disease or pathology, whereby the identified protease is a candidate therapeutic for treatment of the disease or pathology. The Examiner's attention is directed to the instant amendments to claim 1 in which reference to identification of a candidate therapeutic is removed. Basis for the amendment that the method identifies a protease mutein or a catalytically active portion thereof that inactivates an activity of a target protein that is involved with the disease or pathology can be found, for example, on page 21-26, lines 1-3, which states:

> The present invention is drawn to the generation and screening of proteases that cleave proteins known to be involved in disease. The resultant proteins may be used as agents for in vivo therapy.
>
> The invention is broadly drawn to the modification of proteases to alter their substrate sequence specificity, so that the modified proteases cleave a target protein which is involved with or causes a pathology.

Further basis can be found, for example, on page 11, lines 1-3, which states:

> In some examples, the engineered protease is designed to cleave any of the target proteins in Table 1, thereby inactivating the activity of the protein. The protease can be used to treat a pathology associated with that protein, by inactivating one of the target proteins.

and on page 9, lines 17-20, which states:

> If a protease is engineered to recognize a substrate sequence within a target protein or proteins that would *(i.)* alter the function *i.e.* by inactivation of the target protein(s) upon catalysis of peptide bond hydrolysis and, *(ii.)* the target protein(s) are recognized or unrecognized as points of molecular intervention for a particular disease or diseases, then the engineered protease has a therapeutic effect via a proteolysis-mediated inactivation event.

Thus, it respectfully is submitted that claim 1 finds basis in the application as filed, and that the amendments introduced in the Preliminary Amendment and Request for Continued Examination, filed on June 11, 2009, do not introduce new matter.

2. The rejection of claims 1-7, 9, 11-16, 45, 8,50-54, 56-59, 61-63 and 65-78 as allegedly failing to provide a written description for candidate protease mutein that inactivates an activity of a target protein, the inactivation of which ameliorate a disease or condition

The Examiner urges that the specification fails to provide a written description for a candidate protease mutein that inactivates an activity of a target protein the inactivation of which can ameliorate a disease or pathology. The Examiner asserts that

> The Examples do not disclose candidate protease mutants that ameliorate any kind of diseases such as the claim any cancer disease or AIDS (HIV). The detail description provided in Example 8 of the instant specification describes a method of cleaving the different proteins *e.g.*, TNF, VEGR involved in different disease or pathologies. The cleaving enzyme or protein however, has not been shown to be effective in the treatment of the variously claim disease(s). None of the description in the Example describes a disease that has been treated by the candidate enzyme. The specification simply recites the involvement of the specific target protein to the different diseases due to the cleavage of the substrate protein. There is no description as to the candidate protein being useful to treat the disease of at times unknown etiology as cancer or for any types of cancer.

Firstly, the Examiner's attention is directed to the instant amendments of the claims that remove reference to the identification of a candidate therapeutic. Secondly, it respectfully is submitted that the instant claims **are not** directed to either the proteases, nor to methods of treatment of a disease or condition by such proteases; the claims are directed to methods of screening proteases to identify mutants that cleave particular target proteins, target proteins involved in a disease or pathology, such that cleavage inactivates their activity, and by virtue of inactivation of such activity, a disease or pathology could be treated. Thus, a description of the actual *in vivo* treatment is not relevant to the claimed methods, which are screening methods. The instant claims are directed to a **screening method** for identifying from among a library of mutant proteases those that inactivate an activity of a target protein involved in a disease or pathology. The identified proteases then can serve as candidate therapeutics for treating the disease or pathology. In practicing the method, one does not need *a priori* knowledge of a protease that inactivates an activity of a protein, nor knowledge that the protease can treat the disease or condition. That is the point of the screening method. By practice of the method, proteases can be identified that inactivate an activity of a target protein. These proteases can be used as *in vivo* therapeutic agents. Their actual use in methods of treatment is not claimed, and is therefore not relevant to instant claims.

The purpose of the claimed methods is to identify protease muteins that cleave a substrate sequence in a target protein involved with a pathology by identifying those protease mutants from among a library of mutants that have increased cleavage activity and/or altered specificity for a substrate sequence in the target substrate. Cleavage of the target protein by the mutein protease inactivates an activity of the target protein. Thus, protease muteins that inactivate a target protein involved in a disease or pathology are identified. It respectfully is submitted that the specification provides sufficient description of such a method, including description of the target proteins involved in a disease or pathology that can be cleaved by the proteases.

The instant claims are directed to the production and identification of protease muteins that cleave a substrate sequence in a target protein involved with a pathology by identifying those protease mutants from among a library of mutants that have increased cleavage activity and/or altered specificity for a substrate sequence in the target substrate. Cleavage of the target protein by the mutein protease inactivates an activity of the target protein.

The specification provides detailed description for identifying targets whose inactivation could ameliorate (treat) the symptoms of a disease or pathology. For example, on page 23, lines 12-22:

> Proteins **targeted for cleavage and inactivation** are identified by the following criteria: 1) the protein is involved in pathology; 2) there is strong evidence the protein is the critical point of intervention for treating the pathology; 3) proteolytic cleavage of the protein will likely destroy its function. Cleavage sites within target proteins are identified by the following criteria: 1) they are located on the exposed surface of the protein; 2) they are located in regions that are devoid of secondary structure (*i.e.* not in β sheets or α helices), as determined by atomic structure or structure prediction algorithms; (these regions tend to be loops on the surface of proteins or stalks on cell surface receptors); 3) they are located at sites that are likely to inactivate the protein, based on its known function. Cleavage sequences are *e.g.*, four residues in length to match the extended substrate specificity of many serine proteases, but can be longer or shorter.

In addition, at least 45 exemplary target proteins and the disease or pathology in which they are involved are provided in Table 1 on page 9. Therefore, it respectfully is submitted that the specification describes in detail the identifying characteristics of target proteins for screening mutant proteases. In this case the modified proteases are screened for cleavage of the target protein. The specification provides exemplary target proteins, such that one of skill in the art would understand and be able to identify a target protein involved in a pathology or disease.

The specification describes the methods used to identify protease muteins that cleave a substrate sequence in a target protein involved with a pathology by identifying those protease mutants from among a library of mutants that have increased cleavage activity and/or altered specificity for a substrate sequence in the target substrate. The identified muteins are tested for their ability to inactivate, by cleavage, the target protein. It is these **methods** that are the subject of the instant claims, and that are taught in the specification. The methods provide a way to produce and identify the muteins. The claims are not directed to the mutein proteins, or to methods of treatment using the muteins. Thus, demonstration of the actual *in vivo* treatment is irrelevant.

3. The rejection of claims 1-7, 9, 11-16, 45, 8,50-54, 56-59, 61-63 and 65-78 as allegedly failing to provide a written description the genus of proteases encompassed by the method.

The Examiner urges that the genus of proteases employed in the method is too large and structurally diverse. The Examiner states that:

> The specification describes granzyme as the sole cleaving enzyme used in the method. Claim 1, for example, encompass an enormous variation of enzyme muteins, even for granzyme alone which contain numerous allelic variants. It is not known how many allelic variants of granzyme, l*et al*one of the huge protease exist and what the structures even look like for each of any proteases. Thus, the genus of proteases encompassed by the claims as employed in the method is too large and structurally diverse. However, the specification only describes a single species of this genus *e.g.*, granzyme, with particularity but these species are not deemed as representative of said genus.

Again, the Examiner's attention is directed to the fact the claims are directed to a **method of screening a library of mutant proteases**, and not to products. Thus, the genus to which the Examiner refers is a genus of methods that include the elements as recited in the instant claims, not a genus of resulting proteases.

The Examiner's comments appear to be premised on the notion the method can only be performed if one knows all of the structural and functional attributes of the protease to generate protease mutein. This is not correct, and belies the purpose of method as claimed. The point of the method is to identify proteases with altered substrate specificity and/or increased cleavage activity for a target protein, wherein the protease cleaves the target protein and inactivates an activity of the target protein. Hence, the structure/function of the starting protease is not relevant to practice of the method; the method **identifies those protease with the desired function.** Also, the point of a library of mutant proteases is that members of the library are not structurally and functionally representative of each other; rather, the library members necessarily exhibit structural and functional variation in order to permit identification from among the library those that have substrate specificity for the target protein. By following the method, mutant proteases are identified that have increased activity and/or altered substrate specificity for a substrate sequence, such that the protease cleaves and inactivates a target protein containing the sequence. A protease(s) is used as a scaffold for modification; a collection with a variety of modifications is provided and the collection is screened for the ability to cleave a particular target. No structure/function knowledge is needed; the activity can be evolved into the protease.

Notwithstanding this, it respectfully is submitted that the specification provides ample and detailed description of protease scaffolds (including lists, see, *e.g.*, Table 2, of

protease scaffolds) that can be used in the methods. As described in the specification, these scaffolds, regardless of their original structure (*e.g.* sequence) or function (*e.g.* substrate specificity) can be used to generate muteins of the desired specificity and activity, i.e. muteins that cleave and inactivate a target protein involved in a disease or pathology.

The instant claims are directed to methods of identifying a mutein protease that has increased cleavage activity and/or altered substrate specificity for a target protein from among a library of protease muteins, whereby each member of the library has N mutations compared to a wild-type scaffold protease. The specification describes in great detail the scaffold proteases that can be used in the method. The specification describes relevant, identifying characteristics of such proteases, sufficient for use of the proteases in the method as claimed. For example, at page 17, lines 25-32 the specification describes the **process for choosing a scaffold** for use in the method:

> In another embodiment of the invention, scaffold proteases are chosen using the following requirements: 1) The protease is a human or mammalian protease of known sequence; 2) the protease can be manipulated through current molecular biology techniques; 3) the protease can be expressed heterologously at relatively high levels in a suitable host; and 4) the protease can be purified to a chemically competent form at levels sufficient for screening.

In addition, the specification describes exemplary proteases for use in the method. For example, Table 2 sets forth many protease scaffolds that can be used in the method. Notwithstanding this, claims specify the protease used in the method, and thus provide *prima facie* evidence that Applicant's possessed the claimed subject matter. For example, each of claims 7, 53, 61, 63 and 65 recite specific proteases used in the method. Thus, it is eminently clear that Applicant was in possession of a genus of scaffold proteases, including mammalian and human proteases, that can be used in the methods.

II. THE REJECTION OF CLAIMS 1-7, 9, 11-16, 45, 48, 50-54, 56-59, 61-63 AND 65-78 UNDER 35 U.S.C. §112, SECOND PARAGRAPH

Claims 1-7, 9, 11-16, 45, 48, 50-54, 56-59, 61-63 and 65-78 are rejected under 35 U.S.C. §112, second paragraph as being indefinite for various reasons as set forth below. Based on the remarks set forth below, Applicant respectfully requests reconsideration of these rejections.

Relevant law

Claims are not read in a vacuum but instead are considered in light of the specification and the general understanding of the skilled artisan. *Rosemount Inc. v. Beckman Instruments, Inc.*, 727 F.2d 1540, 1547, 221 USPQ 1, 7 (Fed. Cir. 1984), *Caterpillar Tractor Co. v. Berco, S.P.A.*, 714 F.2d 1110, 1116, 219 USPQ 185, 188 (Fed. Cir. 1983). Claim language is satisfactory if it reasonably apprises those of skill in the art of the bounds of the claimed invention and is as precise as the subject matter permits. *Shatterproof Glass Corp. v. Libby-Owens Ford Col.*, 758 F.2d 613, 624, 225 USPQ 634, 641 (Fed. Cir.), cert. dismissed, 106 S.Ct. 340 (1985).

Analysis

1. Claims 1, 53, 59 and 63

Claims 1, 53, 59 and 63 are rejected under 35 U.S.C. §112, second paragraph, as being indefinite because it is alleged that they fail to point out what is included or excluded by the claim. It respectfully is submitted that the claims 1, 53, 59 and 63 clearly recite each step of the method, such that if all of the steps are performed as recited, steps that would be understood by one of skill in the art, then such a method would be encompassed by the claims. Should any one or more of the steps be excluded, then such a method would not be within the scope of the claim. As discussed below with reference to each of the Examiner's particular rejections, it respectfully is submitted that the claims are not indefinite.

2. Claims 1, 53, 59 and 63

Claims 1, 53, 59 and 63 are rejected under 35 U.S.C. §112, second paragraph, as being indefinite because it is alleged that they are "vague and indefinite as to the differentiating features of a disease or pathology in a mammal that is ameliorated by an inactivation of a target protein involved in said disease or pathology."

It respectfully is submitted that one of skill in the art would be able to understand and identify a disease or pathology that is ameliorated by an inactivation of a target protein involved in said disease or pathology. For example, any disease or pathology whose symptoms can be reduced by inactivation of a protein would be included.

As discussed above, the specification provides detailed description of target proteins involved in a disease or pathology. The specification provides detailed description for identifying target whose inactivation could ameliorate (treat) the symptoms of a disease or pathology. For example, on page 23, lines 12-22:

> Proteins **targeted for cleavage and inactivation** are identified by the following criteria: 1) the protein is involved in pathology; 2) there is strong evidence the protein is the

> critical point of intervention for treating the pathology; 3) proteolytic cleavage of the protein will likely destroy its function. Cleavage sites within target proteins are identified by the following criteria: 1) they are located on the exposed surface of the protein; 2) they are located in regions that are devoid of secondary structure (*i.e.* not in β sheets or α helices), as determined by atomic structure or structure prediction algorithms; (these regions tend to be loops on the surface of proteins or stalks on cell surface receptors); 3) they are located at sites that are likely to inactivate the protein, based on its known function. Cleavage sequences are *e.g.*, four residues in length to match the extended substrate specificity of many serine proteases, but can be longer or shorter.

In addition, at least 45 exemplary target proteins and the disease or pathology in which they are involved are provided on page 9, line 26 through page 10, line 31, and in Table 1 on page 12, and pages.

3. Claims 1, 53, 59 and 63

Claims 1, 53, 59 and 63 are rejected under 35 U.S.C. §112, second paragraph, as being indefinite "because the metes and bound of the "catalytically active portions" or a protease scaffold is not clearly set forth in the specification and claims."

Claims 1, 53, 59 and 63 are amended herein to recite "biologically active portions" instead of "catalytically active portions." It respectfully is submitted that a protease scaffold, and a "biologically active portion" thereof, are clearly defined in the specification such that one of skill in the art would readily understand the scope of the claims.

For example, a protease is defined on page 7, line 31-32, as:

> A "protease" is an enzyme that cleaves peptide bonds in proteins.

Further, a protease scaffold is defined on page 2, line 29 through page 3, line 2:

> One embodiment of the invention involves generating a library of protease sequences to be used to screen for modified proteases that cleave a desired target protein at a desired substrate sequence. In one aspect of this embodiment, each member of the library is a protease scaffold with a number of mutations made to each member of the library. A protease scaffold has the same or a similar sequence to a known protease..

On page 8, line 16 and 17, "scaffold" is defined as:

> an existing protease to which various mutations are made. Generally, these mutations changed the specificity and activity of the scaffold [protease].

Thus, a protease scaffold is a protease that is used as a scaffold for mutagenesis. Further, pages 10-16 of the specification are devoted to the identification of a scaffold. As discussed above, the specification details what a protease scaffold is and provides a list (Table 2, which spans pages 12-15) of a variety of exemplary protease scaffolds. In addition, page 16 states:

> Existing proteases are used as scaffolds which include various mutations which change their substrate specificity. Scaffolds can largely include the amino acid sequences of trypsin, chymotrypsin, substilisin, thrombin, plasmin, Factor Xa, urokinase type plasminogen activator (uPA), tissue plasminogen activator (tPA), granzyme B, elastase, papain, cruzain, membrane type serine protease-1 (MTSP-1),

> chymase, neutrophil elastase, granzyme A, plasma kallikrein, granzyme M, complement factor serine proteases, ADAMTS13, neural endopeptidase/neprilysin, and furin or combinations thereof. Preferred scaffolds include granzyme B, MTSP-1, chymase, neutrophil elastase, granzyme A, plasma kallikrein, urokinase type plasminogen activator, granzyme M, chymotrypsin, thrombin, complement factor serine proteases, ADAMTS13, neural endopeptidase/neprilysin, furin, and plasmin.

Thus, it respectfully is submitted that the term "protease scaffold" is not indefinite.

Further, a "biologically active portion" of a protease scaffold is similarly well defined. For example, on page 34, line 18 through page 35, line 3, which describes biologically-active portions of the proteases:

> One aspect of the invention pertains to isolated proteases, and biologically active portions thereof, or derivatives, fragments, analogs or homologs thereof. Also provided are polypeptide fragments suitable for use as immunogens to raise anti protease antibodies. In another embodiment, proteases are produced by recombinant DNA techniques. Alternative to recombinant expression, a protease protein or polypeptide can be synthesized chemically using standard peptide synthesis techniques.
>
> Biologically active portions of protease proteins include peptides comprising amino acid sequences sufficiently homologous to or derived from the amino acid sequences of the protease proteins that include fewer amino acids than the full length protease proteins, and exhibit at least one activity of a protease protein. Typically, biologically active portions comprise a domain or motif with at least one activity of the protease protein. A biologically active portion of a protease protein is a polypeptide which is, for example, 10, 25, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300 or more amino acid residues in length.
>
> Moreover, other biologically active portions of a protein, in which other regions of the protein are deleted, can be prepared by recombinant techniques and evaluated for one or more of the functional activities of a native protease.

Thus, a biologically active portion of a protease is a portion of a protease that exhibits at least one activity of the protease. Thus, it respectfully is submitted that the term "biologically active portion" is not indefinite.

4. Claims 1, 53, 59 and 63

Claims 1, 53, 59 and 63 are rejected under 35 U.S.C. §112, second paragraph, as being indefinite. The Examiner asserts that the "definition of N as a positive integer is infinite and does not circumscribe the claim mutations with particularity in claiming for any kind of residue(s) mutation at any locations or of any length."

As recited in the claims, N is a positive integer. Thus, the mutein protease can have any number of mutations, provided they, as recited in the claims, exhibit increased catalytic activity and/or altered substrate specificity, and cleave a substrate sequence in the target protein, thereby inactivating an activity of the target protein. N cannot be infinite, since the protease scaffolds are not infinite in length. The proteases are used as a scaffold and

modified with any number of mutations to create a collection. Furthermore, the instant claims are directed to methods of identifying protease muteins: it would be contrary to the purpose of the method the number, location and identity of all mutations to be recited. The specification on page 17, lines 16-23, describes the benefits of such a library:

> Also contemplated by the invention are libraries of scaffolds with various mutations that are generated and screened using methods known in the art and those detailed below. Libraries are screened to ascertain the substrate sequence specificity of the members. Libraries of scaffolds are tested for specificity by exposing the members to substrate peptide sequences. The member with the mutations that allow it to cleave the substrate sequence is identified. **The library is constructed with enough variety of mutation in the scaffolds that any substrate peptide sequence is cleaved by a member of the library.** Thus, proteases specific for any target protein can be generated. [emphasis added]

Accordingly, as described in the specification, no knowledge of the number, location and/or identity of the mutations is required to perform the method.

5. Claims 1, 53, 59 and 63

Claims 1, 53, 59 and 63 are rejected under 35 U.S.C. §112, second paragraph, as being indefinite as allegedly being "vague and indefinite with respect to step b) recitation that the members of the library is contacted with a polypeptide comprising a substrate sequence that is present in the target protein." The Examiner asserts that there are no differentiating features of a protein from a polypeptide or a polypeptide comprised in a protein containing a substrate sequence.

Step (b) in, for example, claim 1, recites:

> (b) contacting members of the library with the target protein or with a polypeptide comprising a substrate sequence that is present in the target protein.

The target protein and the substrate sequence in the target protein are clearly introduced previously in the claim, and also described in the specification. It is clear that a target protein contains a substrate sequence, and it is this sequence that is cleaved by the proteases. Further, it is similarly clear from the specification (see *e.g.* page 29, lines 11-16) that a polypeptide other than the target protein, such as a chromogenic peptide, can contain the substrate sequence. Thus, a target protein and/or another polypeptide containing the substrate sequence from the target protein can be used to screen the muteins.

6. Claims 1, 53, 59 and 63

Claims 1, 53, 59 and 63 are rejected under 35 U.S.C. §112, second paragraph, as being indefinite as allegedly being "vague and indefinite in step (d) recitation of "based on

the measured activity and/or specificity" as to the kind of activity or the apparent specificity that can be measured from a protease mutant."

It respectfully is submitted that the limitation in step (d) of "based on the measured activity and/or specificity" refers to the previously introduced limitation in step (c): measuring cleavage activity and/or specificity of at least two members of the library for the target protein or substrate sequence." Thus, it is clear that step (d) recites the base process that is first introduced and recited in step (c).

In addition, the specification clearly describes the methods and assays that can be used to measure the cleavage activity and/or specificity of a protease. For example, page 27, line 31 through page 32 line 26, describes methods for screening the protease for changes in specificity and activity for a substrate sequence and/or target protein. Such methods are exemplified in the Examples, which detail the generation and testing of granzyme B mutants with altered cleavage activity and/or specificity for VEGF and VEGR. Thus, it respectfully is submitted that the limitation in step (d) of "based on the measured activity and/or specificity" is not indefinite.

Further, the Examiner alleges that it is unclear as to how the identified protease is considered to be a "candidate" for treatment of any of disease or pathology. The Examiner asserts that the term "candidate" is a relative term that is not defined by the claim; that the specification does not provide a standard for ascertaining the requisite degree; and that one of ordinary skill in the art would not be reasonably apprised of the scope of the invention. Although Applicant does not agree with the Examiner's assertions, claims 1, 53, 59 and 63 are amended herein to remove recitation that the identified protease is a candidate therapeutic for treatment of the disease or pathology. Thus, the rejection is moot.

III. THE REJECTION OF CLAIMS 1-7, 9, 11-16, 45, 48, 50-54, 56-59, 61-63 AND 65-78 UNDER 35 U.S.C. §102

Claims 1-7, 9, 11-16, 45, 48, 50-54, 56-59, 61-63 and 65-78 are rejected under 35 U.S.C. §102(b) as being anticipated by Lien *et al.*, (Combinatorial Chemistry and High Throughput Screening, 1999)(as evidenced by Shi *et al.*, USP 20020197701). The Examiner asserts that Lien *et al.* discloses all steps of the method as claimed. The Examiner states, however, that Lien *et al.* does not disclose a method of identifying a protease that inactivates a target protein involved in a disease or pathology. The Examiner states that this is implicit in Lien *et al.* based on the teachings of Shi *et al.* (U.S. Patent application No. 20020197701), which states that members of the serine protease family play important roles in cellular

functions that have a demonstrated causative role in diseases. This rejection is respectfully traversed.

Relevant Law

Anticipation requires the disclosure in a single prior art reference of each element of the claim under consideration. *In re Spada*, 15 USPQ2d 1655 (Fed. Cir, 1990), *In re Bond*, 15 USPQ 1566 (Fed. Cir. 1990), *Soundscriber Corp. v. U.S.*, 360 F.2d 954, 148 USPQ 298, 301, adopted 149 USPQ 640 (Ct. Cl.) 1966. See, also, *Richardson v. Suzuki Motor Co.*, 868 F.2d 1226, 1236, 9 USPQ2d 1913,1920 (Fed. Cir.), cert. denied, 110 S.Ct. 154 (1989). "[A]ll limitations in the claims must be found in the reference, since the claims measure the invention." *In re Lang*, 644 F.2d 856, 862, 209 USPQ 288, 293 (CCPA 1981). It is incumbent on Examiner to identify wherein each and every facet of the claimed invention is disclosed in the reference. *Lindemann Maschinen-fabrik Gmbh v. American Hoist and Derrick Co.*, 730 F.2d 1452, 221 USPQ 481 (Fed. Cir. 1984). Further, the reference must describe the invention as claimed sufficiently to have placed a person of ordinary skill in the art in possession of the invention. *In re Oelrich*, 666 F.2d 578, 581, 212 USPQ 323, 326 (CCPA 1981).

The Rejected Claims

The claims are directed to methods for producing and identifying a protease mutein with increased cleavage activity and/or altered substrate specificity for **a target protein involved with a pathology, wherein cleavage of the target protein inactivates the target protein**. For example, independent claim 1, 53, 59 and 63 recite specific steps. The methods include the steps of producing a producing a library of protease muteins of the scaffold, where different member has N mutations relative to the wild-type protease scaffold. Cleavage activity and/or substrate specificity of at least two members of the library for the substrate sequence in the target is measured. At least one mutein protease having an increased cleavage activity and/or altered substrate specificity for cleaving the substrate sequence, relative to the wild-type mammalian protease scaffold is identified. The identified mutein protease is then assessed for **cleavage and inactivation of an activity of the target protein that contains the substrate sequence, thereby identifying a protease mutein or a biologically active portion thereof that inactivates an activity of a target protein that is involved with the disease or pathology**. Dependent claims recite particulars of the methods.

Claim 1 specifies that the target protein is a cell surface molecule that transmits an extracellular signal for cell proliferation, a cytokine, a cytokine receptor and a signaling protein that regulates apoptosis;

Independent claims 53 and dependent claims recite additional steps of the method to identify further mutein proteases. Claim 53 specifies the scaffolds: the human protease scaffold is selected from among a granzyme A, granzyme B, granzyme M, cathepsin, trypsin, chymotrypsin, subtilisin, MTSP-1, elastase, chymase, tryptase, collagenase, papain, neutrophil elastase, complement factor serine proteases, ADAMTS13, neural endopeptidase/neprilysin, furin, cruzain, and urokinase plasminogen activator (uPA);

Claims 59 and 63 (reproduced above) recite particular targets and scaffolds

Hence, each of independent claims 1, 53, 59 and 63 is directed to a method of producing and identifying a protease mutein that cleaves and inactivates a target protein involved in a disease or pathology, including steps of 1) producing a library of protease muteins each having N mutations relative to a wild-type protease; 2) measuring the cleavage activity and/or substrate specificity of at least two members in the library for a substrate sequence in a target protein involved in a disease or pathology, where inactivation of the protein can ameliorate or prevent or treat the disease or pathology; 3) identifying those protease muteins in the library that have an increased cleavage activity and/or altered substrate specificity relative to a wild-type scaffold protease; 4) testing the identified protease(s) for cleavage and inactivation of an activity of the target protein that contains the substrate sequence; and 5) identifying a protease mutein or a biologically active portion thereof that inactivates an activity of a target protein that is involved with the disease or pathology. Each claim recites particular targets and/or scaffolds.

Differences Between the Claims and Disclosure of the Cited References

Lien *et al.*, (Combinatorial Chemistry and High Throughput Screening, 1999, 2:73-90)

Lien *et al.*, is directed to combinatorial mutagenesis methods to generate serine proteases with altered activity. Lien *et al.* is a review article that summarizes various methods to generate serine proteases with altered cleavage specificities, focusing in particular on methods of screening and selection. For example, Lien *et al.* summarizes methods for generating mutant proteases by targeted combinatorial mutagenesis (TCM), such as oligonucleotide mutagenesis; methods for selecting and/or screening for altered cleavage activity; and methods of evaluating cleavage preferences of the mutant proteases. Thus, Lien *et al.*, provides a general overview of the methods that can be used to generate serine

proteases with altered cleavage activity, briefly describing specific examples to illustrate the methods.

According to the methods described in Lien *et al.*, a library of mutant proteases is produced using TCM, as described, for example, on pages 74-75. The cleavage capabilities (activity and specificity) of the mutant proteases is then assessed by screening and/or selection. For example, as described on page 76, the methods can include a selection step and a screening step:

> By eliminating irrelevant or inactive mutants in the TCM library, selection methods obviate the need to manipulate large numbers of clones and yield only mutants that possess the desired cleavage capability. In contrast, screening methods require the investigator to identify active enzymes in the TCM library and assess their individual cleavage capabilities. These two steps are usually done separately, and will be referred to (respectively) as primary and secondary screens. Primary screening of mutant proteases released from bacterial or yeast transformants can be achieved by culturing the library on solid medium containing skim milk. Active proteases (including highly specific ones) hydrolyse the casein causing localised zones of transparency [3, 1 I]. It is also possible to overlay library plates with pre-developed X-ray film and look for zones of clearing caused by digestion of the gelatin [5]. An alternative to primary screening is offered by Venekei *et al.* [5] whose method involves a genetic selection for active mutants (see below). Secondary screening is at its best when a large number of active mutants are tested against a wide range of specific substrates (*e.g.* using a panel of chromogenic substrates that encompasses all possible amino acid residues at the position of interest

The screening and selection methods described in Lien *et al.*, include selection and screening using proteins such as casein, which can be used to select for active proteases, and chromogenic substrates, which can be used to screen for substrate specificity and establish specificity profiles. For example, on page 78:

> Teplayakov *et al.* [8] used TCM to substitute some or all of positions 126-128 in the hydrophobic S4 subsite of subtilase PB92 from *Bacillus acidophilus*. The activities of 22 functional mutants were studied, but using only casein and a chromogenic (amide) tetrapeptide with P1=Phe as substrates.

On page 79:

> The active library mutants were screened using a panel of chromogenic tripeptide (amide) substrates that encompassed 19 different PI residues. From this it was apparent that none of the enzymes were able to cleave at charged residues or at residues that were sterically unusual (such as Pro or D-Ala). The active enzymes could be divided into two groups: one group (14 sequences) with specificities similar to the parental enzyme ALP-M1 9OA, including some with increased overall activity and lowered selectivity, and another group (12 sequences) with lowered activities but altered cleavage capabilities where each enzyme preferred to cleave at Met and His residues.

Thus, the methods described in Lien *et al.* include 1) producing a library of mutant proteases; and 2) selecting and screening the proteases for altered cleavage activity and specificity.

Lien *et al.* does not disclose any target proteins involved in a disease or pathology for which cleavage inactivates the target protein. Further, Lien *et al.* does not disclose a method in which mutant proteases with such activity are produced.

Accordingly, Lien *et al.* discloses methods of producing and identifying protease mutants with altered cleavage activity and specificity, but does not describe a method in which proteases that cleave and inactivate a target protein involved in a disease or pathology. Lien *et al.* does not teach a method that includes each of the steps of 1) producing a library of protease muteins each having N mutations relative to a wild-type protease; 2) measuring the cleavage activity and/or substrate specificity of at least two members in the library for a substrate sequence in the target protein involved in a disease or pathology, where inactivation of the protein can ameliorate or prevent or treat the disease or pathology; 3) identifying those protease muteins in the library that have an increased cleavage activity and/or altered substrate specificity relative to a wild-type scaffold protease; 4) testing the identified protease(s) for cleavage and inactivation of an activity of the target protein that contains the substrate sequence; and 5) identifying a protease mutein or a biologically active portion thereof that inactivates an activity of the target protein that is involved with the disease or pathology..

Lien *et al.*, does not disclose **methods of producing and identifying a protease that inactivates a target protein involved in a disease or pathology**, as provided by the instant claims. The only target proteins described in Lien *et al.*, are those such as casein that can be used to select for any active mutants, and chromogenic substrates that include a substrate sequence, which are used to assess the specificity profile of the mutants. There is no disclosure in Lien *et al.* that the target protein being cleaved in the methods is involved in a disease or pathology nor that cleavage inactivates an activity thereof. Lien *et al.*, does, not, therefore, disclose methods in which the identified protease mutant cleaves a target protein involved in a disease or pathology. Nor is there any disclosure that such inactivation can ameliorate the disease or pathology.

Further, there is no disclosure in Lien *et al.* of a method that includes measuring the cleavage activity and/or substrate specificity of at least two members in the library for a substrate sequence in a target protein involved in a disease or pathology, where inactivation of the protein can ameliorate or prevent or treat the disease or pathology; or identifying those protease muteins in the library that have an increased cleavage activity and/or altered substrate specificity relative to a wild-type scaffold protease; or testing the identified protease(s) for cleavage and inactivation

of an activity of the target protein that contains the substrate sequence; or identifying a protease mutein or a biologically active portion thereof that inactivates an activity of a target protein that is involved with the disease or pathology. Each of these steps is recited in each of independent claims 1, 53, 59 and 63. Lien *et al.* discloses only methods that involve selection and screening of mutant proteases with altered cleavage capabilities; none of the described targets are inactivated and/or involved in a disease or pathology.

In addition, Lien *et al.*, does not disclose the target proteins recites in the claims, such as any of a cell surface molecule that transmits an extracellular signal for cell proliferation, a cytokine, a cytokine receptor and a signaling protein that regulates apoptosis, as recited in claims 1 and 59; or caspase 3, tumor necrosis factor, tumor necrosis factor receptor, interleukin-1, interleukin-1 receptor, interleukin-2, interleukin-2 receptor, interleukin-4, interleukin-4 receptor, interleukin-5, interleukin-5 receptor, interleukin-12, interleukin-12 receptor, interleukin-13, interleukin-13 receptor, p-selectin, p-selectin glycoprotein ligand, Substance P, Bradykinin, PSGL, factor IX, immunoglobulin E, immunoglobulin E receptor, CCR5, CXCR4, glycoprotein 120, glycoprotein 41, hemagglutinin, respiratory syncytium virus fusion protein, B7, CD28, CD2, CD3, CD4, CD40, vascular endothelial growth factor, VEGF receptor, fibroblast growth factor, endothelial growth factor, EGF receptor, TGF receptor, transforming growth factor, Her2, CCR1, CXCR3, CCR2, Src, Akt, Bcl-2, BCR-Abl, glucagon synthase kinase-3, cyclin dependent kinase-2 (cdk-2), and cyclin dependent kinase-4 (cdk-4), such as recited in claim 63.

Analysis

The methods described in Lien *et al.*, are not directed to the identification of a protease that inactivates a target protein involved in a disease or pathology. There is no disclosure in Lien *et al.*, of methods for generating protease muteins that cleave a substrate sequence in a target protein, resulting in inactivation of an activity of the target protein. None of the methods in Lien *et al.*, include a step of testing a mutant protease (or biologically active portion thereof) for cleavage and **inactivation** of an activity of the target protein that contains the substrate sequence, as recited in each of independent claims 1, 53, 59 and 63. Nor is there any disclosure that such a target protein is involved with a disease or pathology in a mammal, such that inactivation of the target protein ameliorates the disease or pathology. Further, Lien *et al.*, does not disclose the target proteins recited in the claims, such as any of a cell surface molecule that transmits an extracellular signal for cell proliferation, a cytokine, a

cytokine receptor and a signaling protein that regulates apoptosis, as recited in claims 1 and 59; or any of the specific target proteins recited in claim 63.

Therefore, Lien *et al.* does not disclose all elements of the claimed methods. Thus, Lien *et al.* does not anticipate any pending claim. Accordingly, for at least these reasons, Lien *et al.* does not anticipate independent claims 1, 53, 59 and 63, or any of the dependent claims therefrom.

Shi *et al.* (U.S. Patent application No. 20020197701)

Shi *et al.*, is directed to the identification of **wild-type** serine proteases and nucleic acids encoding them. Shi *et al.* state, in paragraph [003]:

> Members of the serine protease family which play important roles in a range of cellular functions and which have demonstrated causative roles in human diseases include tissue-type plasminogen activator and thrombin (thrombosis and blood clotting), urokinase-type plasminogen activator (cancer and metastasis), trypsin and elastase (emphysema and liver disease) and angiotensin converting enzyme (hypertension).

Shi *et al.* is directed to the identification of wild-type serine proteases, which, as noted in the application, can play a role in human diseases. Shi *et al.* is **not** directed to methods of generating mutant proteases, particularly those that are mutated to inactivate an activity of a target protein involved with a disease or pathology in a mammal. There is no disclosure in Shi *et al.* of any methods that include any one or more of 1) producing a library of protease muteins each having N mutations relative to a wild-type protease; 2) measuring the cleavage activity and/or substrate specificity of at least two members in the library for a substrate sequence in a target protein involved in a disease or pathology, where inactivation of the protein can ameliorate or prevent or treat the disease or pathology; 3) identifying those protease muteins in the library that have an increased cleavage activity and/or altered substrate specificity relative to a wild-type scaffold protease; 4) testing the identified protease(s) for cleavage and inactivation of an activity of the target protein that contains the substrate sequence; and 5) identifying a protease mutein or a biologically active portion thereof that inactivates an activity of a target protein that is involved with the disease or pathology.

Analysis

Shi *et al.* is directed to the identification of wild-type serine proteases. Shi *et al.*, does not disclose a method that includes any of the steps of the instantly claimed methods. There is no disclosure in Shi *et al.* of any methods that include any one or more of 1) producing a library of protease muteins each having N mutations relative to a wild-type protease; 2) measuring the cleavage activity and/or substrate specificity of at least two members in the library for a substrate sequence in a target protein involved in a disease or pathology, where inactivation of the

protein can ameliorate or prevent or treat the disease or pathology; 3) identifying those protease muteins in the library that have an increased cleavage activity and/or altered substrate specificity relative to a wild-type scaffold protease; 4) testing the identified protease(s) for cleavage and inactivation of an activity of the target protein that contains the substrate sequence; and 5) identifying a protease mutein or a biologically active portion thereof that inactivates an activity of a target protein that is involved with the disease or pathology. Shi *et al.* describes the identification of wild type serine proteases and contains comments regarding the involvement of wild-type serine proteases in cellular function and disease. Thus, Shi *et al.* is not relevant to the instant claims and does not anticipate any claim.

Rebuttal to the Examiner's comments

The Examiner asserts that the limitation of inactivation of a target protein involved in a disease or pathology in a mammal by the mutein proteases of the method, such that the disease or pathology is ameliorated, is inherent in Lien *et al.*, as demonstrated by Shi *et al.* Specifically, the Examiner alleges:

> The claim inactivation of a target protein involved with a disease or pathology in a mammal that ameliorate a disease is a property inherent or implicit to the teachings of Lien. [This is evident from the teachings of Shi *et al* at *e.g.*, paragraph [0003]. Shi states that members of the serine protease family which play important roles in a range of cellular functions and which have demonstrated causative roles in human diseases include tissue-type plasminogen activator and thrombin (thrombosis and blood clotting), urokinase-type plasminogen activator (cancer and metastasis), trypsin and elastase (emphysema and liver disease) and angiotensin converting enzyme (hypertension).

It respectfully is submitted that the Examiner has either misread the claims or misread Shi *et al.* The instant claims are directed to **methods of identifying protease muteins that inactivate a target protein involved in a disease or pathology**. Such inactivation can ameliorate the disease or pathology. Thus, the method is directed to the generation of mutant proteases. These proteases cleave, and thereby inactivate, a target protein that itself is involved in the disease or pathology. Thus, it is not the protease mutein that is involved in the disease or pathology, but the target protein.

As discussed above, Shi *et al.* is directed to the identification of wild-type serine proteases. Shi *et al.* does not disclose any methods of producing and identifying mutant proteases that cleave and inactivate a target protein. Shi *et al.* states that the serine proteases can be involved in cellular functions and disease. **Shi *et al.* is not referring to a target protein, but instead to the serine protease**. Thus, Shi *et al.* does not address, in any way, the limitation in the claims that the mutein protease inactivates a target protein involved in a disease, thereby ameliorating the disease.

As discussed above, Lien *et al.* discloses methods that include 1) producing a library of mutant proteases; and 2) selecting and screening the proteases for altered cleavage activity and specificity. Accordingly, Lien *et al.* discloses methods of producing and identifying protease mutants with altered cleavage activity and specificity. Lien *et al.* does not disclose any methods that include a step of testing a mutant protease (or biologically active portion thereof) for cleavage and **inactivation** of an activity of a target protein, as recited in each of independent claims 1, 53, 59 and 63. Nor does Lien *et al.* disclose methods in which the target protein used in the screening steps of the method is involved in a disease or pathology.

There is nothing in Shi *et al.* that evidences that either of these limitations that are lacking in Lien *et al.* is inherent in the teachings of Lien *et al.* Shi *et al.* states that the wild type serine proteases that are the subject of the application can be involved in cellular functions and disease. Not only does Shi *et al.* not disclose any methods of producing and identifying mutant proteases that cleave and inactivate a target protein, but in the statements quoted by the Examiner, Shi *et al.* **is not referring to a target protein, but instead to the serine protease**. Thus, neither Lien *et al.* or Shi *et al.*, alone or combination, disclose the instant methods, which are directed to methods of producing and identifying mutant proteases that cleave and inactivate a target protein involved in a disease or pathology.

Accordingly, it respectfully is submitted that Lien *et al.* as supported by Shi *et al.* does not anticipate independent claims 1, 53, 59 and 63, or any of the dependent claims therefrom. Thus, claims 1-7, 9, 11-16, 45, 48, 50-54, 56-59, 61-63 and 65-78 are not anticipated by Lien *et al.*, as supported by Shi *et al.*

IV. THE REJECTION OF CLAIMS 1-7, 9, 11-16, 45, 48, 50-54, 56-59, 61-63 AND 65-78 UNDER 35 U.S.C. §102

Claims 1-5, 7, 9, 11, 13-16, 48, 50-54, 58-59, 61-63, 65 and 67 are rejected under 35 U.S.C. §102(b) as being anticipated by Guinto *et al.* (*Proc. Natl. Acad. Sci. U.S.A.* 96:1852-1857 (1999). The Examiner alleges that Guinto *et al.* discloses mutants of Factor VII at position 225 that were contacted with substrates of fibrinogen, protein C and antithrombin III, and mutant proteases identified that influence catalytic activity. The Examiner states that catalysis of the target fibrinogen or protein C will result in inactivation of the target. This rejection is respectfully traversed.

Relevant Law

See related section above.

The rejected claims

The claims are discussed in a related section above.

Differences Between the Claims and Disclosure of Guinto *et al.*

Guinto *et al.* is directed to the role of the amino acid residue at position 225 in serine proteases. Guinto *et al.* describes studies involving saturation mutagenesis of thrombin at position 225. The resulting mutants were then tested for their ability hydrolyze a chromogenic substrate, fibrinogen and protein C, as well as their ability to be inhibited by antithrombin III. These are the only target proteins disclosed in Guinto *et al.* **None of these target proteins are inactivated when cleaved by the mutant thrombin proteases**, as required by the instant claims. For example, thrombin cleaves fibrinogen to produce fibrinopeptide A and fibrinopeptide B, which assemble into fibrin to form a clot. Thus, rather than being inactivated by thrombin, fibrinogen is activated to form a clot following cleavage by thrombin. Similarly, cleavage of protein C by thrombin (when complexed with thrombomodulin) results in activation of protein C, not inactivation. Antithrombin III is an inhibitor of thrombin, and thus inactivates thrombin. It is not inactivated by thrombin.

Analysis

Guinto *et al.* describes the saturation mutagenesis of thrombin at position 225 and subsequent screening of the mutants for cleavage activity for a chromogenic substrate, fibrinogen and protein C, as well as their ability to be inhibited by antithrombin III. Guinto *et al.* does not disclose making a library of protease mutants, nor does it disclose testing the identified proteases that have increased cleavage activity or altered substrate specificity or the ability to cleave and inactivate an activity of a target protein. Thus, at most, Guinto *et al.* discloses methods of making modified proteases.

Guinto *et al.* **does not** disclose methods of generating protease muteins that cleave a substrate sequence in a target protein, resulting in inactivation of the target protein, as recited in each of the instant claims. There is no disclosure in Guinto *et al.* of methods for generating mutants that cleave and inactivate a target protein. The target proteins cleaved by the mutant thrombin proteases in Guinto *et al.* **are activated, not inactivated.** Further, any disease or pathology that might be associated with these target proteins can not be ameliorated by inactivation of them by the mutant thrombin proteases, as no inactivation occurs. Thus, Guinto *et al.* does not disclose at least two important limitations in the methods of the instant claims 1) cleavage of a substrate sequence in the target protein inactivates an activity of the target protein and 2) inactivation of the target protein by the mutein protease

can ameliorate a disease or pathology. Further, Guinto *et al.* does not disclose a method that includes: 1) producing a library of protease muteins each having N mutations relative to a wild-type protease; 2) measuring the cleavage activity and/or substrate specificity of at least two members in the library for a substrate sequence in a target protein involved in a disease or pathology, where inactivation of the protein can ameliorate or prevent or treat the disease or pathology (cleavage of the "target" proteins in Guinto *et al.*, **does not** result in inactivation thereof) ; 3) identifying those protease muteins in the library that have an increased cleavage activity and/or altered substrate specificity relative to a wild-type scaffold protease; 4) testing the identified protease(s) for cleavage and inactivation of an activity of the target protein that contains the substrate sequence; and 5) identifying a protease mutein or a biologically active portion thereof that inactivates an activity of a target protein that is involved with the disease or pathology.

Further, Guinto *et al.* only discloses fibrinogen, protein C, and antithrombin III as target proteins. None of these are a cell surface molecule that transmits an extracellular signal for cell proliferation, a cytokine, a cytokine receptor and a signaling protein that regulates apoptosis, as recited in claims 1 and 59. Nor are they included among the target proteins recited in claim 63. Thus, Guinto *et al.* fails to disclose at least three limitations of claims 1, 59 and 63, and dependent claims therefrom, and at least two limitations of claim 53 and dependent claims therefrom. Accordingly, Guinto *et al.* does not anticipate claims 1-7, 9, 11-16, 45, 48, 50-54, 56-59, 61-63 and 65-78 are not anticipated by Guinto *et al.*

V. THE REJECTION OF CLAIMS 1-7, 9, 11-16, 45, 48, 50-54, 56-59, 61-63 and 65-78 UNDER 35 U.S.C. §103

Claims 1-7, 9, 11-16, 45, 48, 50-54, 56-59, 61-63 and 65-78 are rejected under 35 U.S.C. §103(a) as being unpatentable over Lien *et al.*, (Combinatorial Chemistry and High Throughput Screening, 1999, 2:73-90) in light of either Harris *et al.* I (*J. Biol. Chem.*, 273: 27364-27373 (1998)) or Harris *et al.* II (Harris *et al.* II (Current Opinion in Chemical Biology, 2:127-132 (1998)) and Waugh *et al.* (Nature Structure Biology). The Examiner alleges that Lien *et al.*, teaches a method of identifying mutein proteases with altered substrate specificity or cleavage activity, but does not disclose the protease as granzyme B and the substrate as caspase, the elected species. The Examiner asserts that Harris *et al.* I, Harris *et al.* II and Waugh *et al.* teaches the use of granzyme B for cleavage of caspase. The Examiner concludes that it would have been obvious to one of ordinary skill to have used granzyme as

the enzyme as taught by Harris *et al.* I, Harris *et al.* II and Waugh *et al.* in the method of Lien *et al.* This rejection is respectfully is traversed.

Relevant Law

To establish prima facie obviousness under 35 U.S.C. §103, all the claim limitations must be taught or suggested by the prior art. In re Royka, 490 F.2d 981, 180 USPQ 580 (CCPA 1974). This principle of U.S. law regarding obviousness was not altered by the recent Supreme Court holding in *KSR International Co. v. Teleflex Inc.,* 127 S.Ct. 1727, 82 USPQ2d 1385 (2007). In KSR, the Supreme Court stated that "Section 103 forbids issuance of a patent when 'the differences between the subject matter sought to be patented and the prior art are such the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains.'" *KSR Int'l Co. v. Teleflex Inc.*, 127 S.Ct. 1727, 1734, 82 USPQ2d 1385, 1391 (2007).

The question of obviousness is resolved on the basis of underlying factual determinations including (1) the scope and content of the prior art, (2) any differences between the claimed subject matter and the prior art, (3) the level of skill in the art. *Graham v. John Deere Co.,* 383 U.S. 1, 17-18, 148 USPQ 459, 467 (1966). See also *KSR*, 127 S.Ct. at 1734, 82 USPQ2d at 1391 ("While the sequence of these questions might be reordered in any particular case, the [Graham] factors continue to define the inquiry that controls.") The Court in Graham noted that evidence of secondary considerations, such as commercial success, long felt but unsolved needs, failure of others, etc., "might be utilized to give light to the circumstances surrounding the origin of the subject matter sought to be patented." 383 U.S. at 18, 148 USPQ at 467. Furthermore, the Court in KSR took the opportunity to reiterate a second long-standing principle of U.S. law: that a holding of obviousness requires the fact finder (here, the Examiner), to make explicit the analysis supporting a rejection under 35 U.S.C. 103, stating that "rejections on obviousness cannot be sustained by mere conclusory statements; instead, there must be some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness. *Id.* at 1740-41, 82 USPQ2d at 1396 (citing *In re Kahn*, 441 F.3d 977, 988, 78 USPQ2d 1329, 1336 (Fed. Cir. 2006)).

While the KSR Court rejected a rigid application of the teaching, suggestion, or motivation ("TSM") test in an obviousness inquiry, the Court acknowledged the importance of identifying "a reason that would have prompted a person of ordinary skill in the relevant field to combine the elements in the way the claimed new invention does" in an obviousness determination. *KSR*, 127 S. Ct. at 1731. The court stated in dicta that, where there is a

"market pressure to solve a problem and there are a finite number of identified, predictable solutions, a person of ordinary skill has good reason to pursue the known options within his or her technical grasp. If this leads to the anticipated success, it is likely the product not of innovation but of ordinary skill and common sense. In that instance the fact that a combination was obvious to try **might** show that it was obvious under § 103."

In a post-KSR decision, *PharmaStem Therapeutics. Inc. v. ViaCell. Inc.*, 491 F.3d 1342 (Fed. Cir. 2007), the Federal Circuit stated that:

> an invention would not be invalid for obviousness if the inventor would have been motivated to vary all parameters or try each of numerous possible choices until one possibly arrived at a successful result, where the prior art gave either no indication of which parameters were critical or no direction as to which of many possible choices is likely to be successful. Likewise, an invention would not be deemed obvious if all that was suggested was to explore a new technology or general approach that seemed to be a promising field of experimentation, where the prior art gave only general guidance as to the particular form of the claimed invention or how to achieve it.

Furthermore, KSR has not overruled See *In re Papesch*, (315 F.2d 381, 137 USPQ 43 (CCPA 1963)), *In re Dillon*, 919 F.2d 688, 16 USPQ2d 1897 (Fed. Cir. 1991), and *In re Deuel* (51 F.3d 1552, 1558-59, 34 USPQ2d 1210, 1215 (Fed. Cir. 1995)). "In cases involving new compounds, it remains necessary to identify some reason that would have led a chemist to modify a known compound in a particular manner to establish prima facie obviousness of a new claimed compound." *Takeda v. Alphapharm*, 492 F.3d 1350 (Fed. Cir. 2007).

The mere fact that prior art may be modified to produce the claimed product does not make the modification obvious unless the prior art suggests the desirability of the modification. *In re Fritch*, 23 U.S.P.Q.2d 1780 (Fed. Cir. 1992); see, also, *In re Papesh*, 315 F.2d 381, 137 U.S.P.Q. 43 (CCPA 1963). In addition, if the proposed modification or combination of the prior art would change the principle of operation of the prior art invention being modified, then the teachings of the references are not sufficient to render the claims prima facie obvious. *In re Ratti*, 270 F.2d 810, 123 USPQ 349 (CCPA 1959).

As always, unexpected properties must always be considered in the determination of obviousness. A compound's structure and properties are inseparable so that unexpected properties are part of the subject matter as a whole. *In re Papesch*, 315 F.2d 381, 391, 137 USPQ 43, 51 (CCPA 1963)

The disclosure of the applicant cannot be used to hunt through the prior art for the claimed elements and then combine them as claimed. *In re Laskowski*, 871 F.2d 115, 117, 10

USPQ2d 1397, 1398 (Fed. Cir. 1989). "To imbue one of ordinary skill in the art with knowledge of the invention in suit, when no prior art reference or references of record convey or suggest that knowledge, is to fall victim to the insidious effect of a hindsight syndrome wherein that which only the inventor taught is used against its teacher" *W.L. Gore & Associates, Inc. v. Garlock Inc.*, 721 F.2d 1540, 1553, 220 USPQ 303, 312-13 (Fed. Cir. 1983).

The rejected claims

The claims are discussed in a related section above.

Differences Between the Claims and Teachings of the Cited References

Lien *et al.*, (Combinatorial Chemistry and High Throughput Screening, 1999, 2:73-90)

As discussed above, Lien *et al.*, is directed to combinatorial mutagenesis methods to generate serine proteases with altered activity. Lien *et al.* is a review article that summarizes various methods to generate serine proteases with altered cleavage specificities, focusing in particular on methods of screening and selection. For example, Lien *et al.* summarizes methods for generating mutant proteases by targeted combinatorial mutagenesis (TCM), such as oligonucleotide mutagenesis; methods for selecting and/or screening for altered cleavage activity; and methods of evaluating cleavage preferences of the mutant proteases, such as . Thus, Lien *et al.*, provides a general overview of the methods that can be used to generate serine proteases with altered cleavage activity or substrate specificity, briefly describing specific examples to illustrate the methods. Lien *et al.* teaches methods of generating serine proteases with altered substrate specificity using libraries of mutant proteases and screening the libraries for cleavage activity. This, however, is the full extent of the teachings of Lien *et al.*

Lien *et al.*, **does not disclose or teach methods of identifying a protease that inactivates a target protein involved in a disease or pathology**, as recited in the instant claims. None of the methods in Lien *et al.* a) include a step of testing a mutant protease (or biologically active portion thereof) for cleavage and **inactivation** of an activity of the target protein that contains the substrate sequence nor does Lien *et al.* disclose a method in which mutant proteases the cleave and inactivate a target protein involved in a disease or pathology are identified. Nor is there any disclosure in Lien *et al.* that b) the target protein used in the methods is involved with a disease or pathology in a mammal, or that c) such inactivation of the target protein ameliorates the disease or pathology.

As discussed above, Lien *et al.* does not disclose, teach or suggest methods in which mutant proteases are identified that cleave and inactivate an activity of a target protein involved in a disease or pathology, nor does Lien *et al.* teach or suggest a method that includes steps in which cleavage activity and/or substrate specificity of at least two members in a library of mutant proteases (or biologically active portions thereof) for a substrate sequence in a target protein involved in a disease or pathology, where inactivation of the protein can ameliorate or prevent or treat the disease or pathology, is measured; or in which protease muteins in the library that have an increased cleavage activity and/or altered substrate specificity relative to a wild-type scaffold protease for such sequence in such target protein are identified and then tested for cleavage and inactivation of an activity of the target protein that contains the substrate sequence, or in which a protease mutein or a biologically active portion thereof that inactivates an activity of a target protein that is involved with the disease or pathology is identified. As discussed above, contrary to the Examiner's assertion, Shi *et al.* does not describe any of these limitations nor show that they are taught by Lien *et al.*, As discussed below, none of Harris *et al.* I, Harris *et al.* II or Waugh *et al.*, singly or in any combination thereof, cures these deficiencies.

Harris *et al.* I (*J. Biol. Chem.*, 273: 27364-27373 (1998

Harris *et al.* I is directed to the screening combinatorial **substrate** libraries to identify *in vivo* substrates of rat granzyme B in order elucidate its substrate specificity. Using the combinatorial substrate libraries, Harris *et al.* I teaches identification of the optimal P4-P2 substrate specificity profile of granzyme B. Harris *et al.* I teaches the optimal P4 to P2' granzyme B cleavage site to be (Ile>Val)(Glu>Gln= Met)Xaa-Asp/ Xaa-Gly, for example, the substrate sequence IEPD. Harris *et al.* also teaches identification of *in vivo* targets of granzyme B based on the elucidation of the substrate specificity of granzyme B. For example, Harris *et al.* teaches that based on the substrate specificity of granzyme B, certain caspases (caspases 3 and 7) , based on their sequences, are more likely substrates then other caspases. Harris *et al.*, also teaches that based on the sequence specificity of granzyme B, nuclear lamin A and nuclear poly(ADP)ribose polymerase (PARP) are potential *in vivo* substrates for granzyme B. Harris *et al.* I also teaches that amino acid position Arginine 192 is a structural determinant of specificity of granzyme B, since granzyme B mutations R192E and R192A exhibit **reduced** hydrolysis of the optimal tetrapeptide substrate Ac-IEPD-AMC and non-optimal tetrapeptide substrate Ac-IKPD-AMC compared to the wild-type enzyme.

Harris *et al.* is of no relevance to the instant claims, since it fails to teach or suggest **any** element of the instantly claimed methods. For example, Harris *et al.* does not teach modification of substrate specificity, nor a method that includes providing a library of modified proteases based on a particular scaffold, as required by the instant claims. Harris *et al.* I does not teach or suggest a method of identifying mutein proteases, including mutein granzyme B proteases, that have increased cleavage activity and/or altered specificity for a target protein involved in a pathology.

Particularly, Harris *et al.* **does not teach or suggest a method of producing and identifying mutein proteases, such as mutein granzyme B proteases, that cleave a substrate sequence in a target protein, thereby inactivating the target protein**. There is no suggestion in Harris *et al.* of a method that includes a step of testing a mutant protease for cleavage and inactivation of an activity of a target protein. Nor does Harris *et al.* teach that by inactivating the target protein, the disease or condition associated with that target protein can be ameliorated. Harris *et al.* suggests that the mutant granzyme are more likely to use some caspases as substrates then other caspases. Cleavage of caspases by granzyme B activates the caspases. There is no teaching or suggestion in Harris *et al.* that the mutant granzyme B proteases inactivate any caspases. Hence, Harris *et al.* fails to cure the deficiencies in the teachings of Lien *et al.*

Harris *et al.* II (Current Opinion in Chemical Biology, 2:127-132 (1998))

Harris *et al.* II is a review article directed to methods for determinants of protease specificity and for modifying specificity as a means to "understand the factors involved in protease specificity." Harris *et al.* II teaches that several methods that have been employed to make changes in enzyme specificity in order to understand protein design principles. The first method, designated rational redesign specificity, requires a detailed understanding of the catalytic mechanism and sequence determinants for a particular protease as basis for predictably altering specificity. Harris *et al.* II also teaches complete random mutagenesis and states that this requires large libraries of mutants to identify a desired function. Another method, employs comparative analysis using homologous proteins that differ in substrate specificity. In another method, describes the use of alanine scanning to identify residues involved in activity, followed by replacement of identified residues with all 19 amino acids to produce modified proteins with altered activity. In a final method, Harris *et al.* teaches using directed evolution, such as DNA shuffling methods, to generate enzymes with modified activities.

Harris *et al.* II is a review article that describes methods that have been used to identify structural determinants. None of the methods described by Harris *et al.* II are for generating a protease that has specificity for a particular target involved in a pathology. Further, none of the methods are for generating proteases that cleave a target protein, thereby inactivating the target protein, or that such activation can ameliorate a disease or pathology associated with target protein. None of the methods include the steps required by the instant claims. The methods described by Harris *et al.* II provides a general review of the state of the art at the time of its publication.

Harris *et al.* II does not teach or suggest a method of producing and identifying a protease with increased cleavage activity and/or altered substrate specificity for a target protein involved in a pathology, wherein cleavage of the target protein inactivates an activity of the target protein. Nor does Harris *et al.* II teach or suggest that such activation can ameliorate a disease or pathology associated with target protein. Hence, Harris *et al.* II fails to teach or suggest the elements of the instantly claimed methods, particularly those elements pertaining to the cleavage and inactivation of the target protein involved in a disease or pathology, such that the disease or pathology is ameliorated. Thus, the combination of Harris *et al.* II and Lien *et al.* fail to teach or suggest each of the elements of the instantly claimed methods.

Waugh *et al.* (Nature Structure Biology 7: 762-765 (2000)

Waugh *et al.* teaches that granzymes are involved in inducing apoptosis by acting on downstream substrates such as caspases by ***activation*** cleavage. Waugh *et al.* teaches that cleavage of caspases by granzymes result in **activation of the substrate**. Waugh *et al.* further describes the structure of granzyme B, and elucidation of the molecular determinants of specificity therefrom. Waugh *et al.* teaches the residues in granzyme B that play a role in determining substrate specificity as deduced from a three dimensional structure of granzyme B in complex with a macromolecular inhibitor, ecotin.

Waugh *et al.* does not teach or suggest any of the instantly claimed methods. Particularly, Waugh *et al.* does not teach or suggest the elements of the methods as claimed for which Lien *et al.* is deficient. For example, there is no teaching or suggestion in Waugh *et al.*, as there is no teaching or suggestion in Lien *et al.*, of a method of evolving granzyme B or other proteases to identify mutants that cleave a substrate sequence in a target protein, **thereby inactivating the target protein**. Waugh *et al.* does not teach or suggest, like Lien *et al.* does not teach or suggest, any method that includes a step of testing a protease for

cleavage and inactivation of an activity of a target protein that is involved in a disease or pathology. Waugh *et al.* teaches that granzyme cleaves substrates (*e.g.* caspases) in order to **activate** them. There is no teaching or suggestion of any method in which granzymes, including granzyme B or any other protease, is modified to inactivate a substrate involved in a disease or pathology, thereby serving as a treatment of the pathology. Hence, Waugh *et al.* fails to cure the deficiencies in the teachings of Lien *et al.*

The combination of teachings of the cited references does not result in the instantly claimed methods.

The Examiner has failed to set forth a prima facie case of obviousness because none of Lien *et al.*, Harris *et al.* I, Harris *et al.* II and/or Waugh *et al.*, singly or in any combination thereof teaches all elements as claimed. Each of independent claims 1, 53, 59 and 63 is directed to a method of producing and identifying a protease mutein that cleaves and inactivates a target protein involved in a disease or pathology, including steps of 1) producing a library of protease muteins each having N mutations relative to a wild-type protease; 2) measuring the cleavage activity and/or substrate specificity of at least two members in the library for a substrate sequence in a target protein involved in a disease or pathology, where inactivation of the protein can ameliorate or prevent or treat the disease or pathology; 3) identifying those protease muteins in the library that have an increased cleavage activity and/or altered substrate specificity relative to a wild-type scaffold protease; 4) testing the identified protease(s) for cleavage and inactivation of an activity of the target protein that contains the substrate sequence; and 5) identifying a protease mutein or a biologically active portion thereof that inactivates an activity of a target protein that is involved with the disease or pathology The cited references, singly or in any combination thereof, teaches or suggests such a method.

Lien *et al.* teaches methods of generating serine proteases with altered substrate specificity using libraries of mutant proteases and screening the libraries for cleavage activity. This, however, is the full extent of the teachings of Lien *et al.* Lien *et al.* does not teach or suggest methods of producing and identifying a protease mutein **that cleaves and inactivates a target protein involved in a disease or pathology**. None of the methods in Lien *et al.* a) include a step of testing a mutant protease (or biologically active portion thereof) for cleavage and **inactivation** of an activity of the target protein that contains the substrate sequence nor identifying a mutant protease with such activity, as recited in each of independent claims 1, 53, 59 and 63. Nor is there any disclosure in Lien *et al.* that b) the target protein used in the methods is involved with a disease or pathology in a mammal, or

that c) such inactivation of the target protein ameliorates the disease or pathology. None of Harris *et al.* I, Harris *et al.* II and/or Waugh *et al.*, singly or in any combination thereof, cures these deficiencies.

As discussed above, Harris *et al.* I teaches elucidation of the substrate specificity of wild-type rat granzyme B, and the putative identification of *in vivo* substrates based on knowledge of the substrate specificity. There is no teaching or suggestion for using granzyme B as a scaffold produce a mutein protease with increased cleavage activity and/or altered its substrate specificity for a target, such that the mutein **inactivates an activity of the target protein, nor any methods for doing so**. Nor is there any suggestion that inactivation of the target protein bu cleavage with the mutein protease can ameliorate the disease or condition that the target protein is involved in. Harris *et al.* I, in the abstract, states that: I states that:

> Mutagenesis of arginine 192 to glutamate reversed the preference for negatively charged amino acids at P3 to positively charged amino acids. The preferred substrate sequence matches the **activation** sites of caspase 3 and caspase 7 and thus is consistent with the role of granzyme B in activation of these proteases during apoptosis. [emphasis added]

The Examiner herself notes, on page 21, line 1 of the Office Action, that Harris *et al.* I teaches that granzyme B cleaves and **activates** several caspases involved in apoptosis. Harris *et al.* II does not, therefore, teach or suggest a method that a) includes a step of testing a mutant protease (or biologically active portion thereof) for cleavage and inactivation of an activity of a target protein; or b) the target protein used in the methods is involved with a disease or pathology in a mammal, or that c) such inactivation of the target protein ameliorates the disease or pathology. Thus, Harris *et al.* I does not cure the deficiencies of Lien *et al.*

Harris *et al.* II does not cure the deficiencies in Lien *et al.* or Harris *et al.* I. Harris *et al.* II is a general review of the art of engineering proteases. Harris *et al.* II teaches various known methods of engineering proteases. None of the teachings in Harris *et al.* II teach or suggest any elements of the claimed methods nor any methods of producing and identifying protease muteins that cleave and inactivate a target protein in a pathology, by identifying protease muteins that have increased cleavage activity and/or altered substrate specificity for a substrate sequence in the target protein. The Examiner asserts on page 21 of the Office Action that "Harris II throughout the article, at *e.g.*, pages 127-129, basically discloses the same method as Harris (I)." Thus, the Examiner appears to acknowledge that Harris *et al.* II does not provide any relevant teaching beyond that provided by Harris *et al.* I. As noted above, Harris *et al.* I. does not in any way teach or suggest the claimed methods. Similarly, nor does Harris *et al.* II. In particular, Harris *et al.* II does not teach or suggest a method that a) includes a step of testing a mutant protease (or

biologically active portion thereof) for cleavage and inactivation of an activity of the target protein that contains the substrate sequence; or b) the target protein used in the methods is involved with a disease or pathology in a mammal, or that c) such inactivation of the target protein ameliorates the disease or pathology, as recited in claims 1, 53, 59 and 63. Accordingly, for Harris *et al.* II does not cure the deficiencies in Lien *et al.*

Waugh *et al.* describes the structure of granzyme B, and elucidation of the molecular determinants of specificity therefrom. Waugh *et al.* teaches that granzymes are involved in inducing apoptosis by acting on downstream substrates such as caspases by **activation** cleavage. Waugh *et al.* teaches that cleavage of caspases by granzymes result in **activation** of the substrate. Waugh *et al.* does not teach or suggest any method of producing and identifying a mutant protease that cleaves and inactivates target protein involved in a disease or pathology. Particularly, Waugh *et al.* does not teach or suggest any elements of the method as claimed for which Lien *et al.*, Harris *et al.* I and/or Harris *et al.* II are deficient. For example, Waugh *et al.* does not teach or suggest a method of that a) includes a step of testing a mutant protease (or biologically active portion thereof) for cleavage and inactivation of an activity of the target protein that contains the substrate sequence; or b) the target protein used in the methods is involved with a disease or pathology in a mammal, or that c) such inactivation of the target protein ameliorates the disease or pathology. Hence, Waugh *et al.* fails to cure the deficiencies in the teachings of Lien *et al.*

Lien *et al.* teaches only methods of generating serine proteases with altered substrate specificity using libraries of mutant proteases and screening the libraries for cleavage activity. None of the methods in Lien *et al.* a) include a step of testing a mutant protease (or biologically active portion thereof) for cleavage and **inactivation** of an activity of the target protein that contains the substrate sequence; b) require that the target protein used in the methods is involved with a disease or pathology in a mammal, or c) require that such inactivation of the target protein ameliorates the disease or pathology. None of Harris *et al.* I, Harris *et al.* II and/or Waugh *et al.*, singly or in any combination thereof, cure any of these deficiencies.

Thus, the combination of teachings of the cited references fails to teach or suggest each of the elements of the independent claims, including: 1) producing a library of protease muteins each having N mutations relative to a wild-type protease; 2) measuring the cleavage activity and/or substrate specificity of at least two members in the library for a substrate sequence in a target protein involved in a disease or pathology, whereby cleavage of the

substrate sequence in the target protein inactivates the target protein; 3) identifying those protease muteins in the library that have an increased cleavage activity and/or altered substrate specificity relative to a wild-type scaffold protease; 4) testing the identified protease(s) for cleavage and inactivation of an activity of the target protein that contains the substrate sequence; and 5) identifying protease mutein or a biologically active portion thereof that inactivates an activity of a target protein that is involved with the disease or pathology. Since a *prima facie* case of obviousness requires that the references, singly or in any combination, teach or suggest all elements as claimed, the Examiner has failed to set forth a case of *prima facie* obviousness.

V. THE REJECTION OF THE CLAIMS FOR NONSTATUTORY DOUBLE PATENTING

Claims 1-7, 9, 11-16, 45, 48, 50-54, 56-59, 61-63 and 65, as amended, are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-10, for example, of copending Application No. 12/005949 ('949 application). Although the conflicting claims are not identical, they are not patentably distinct from each other because the instant claim method is similar, if not nearly identical to the method of the '949 application. The subject matter of the instant and the '949 applications overlap in scope. Deferral of resolution of this rejection respectfully is requested.

It is not possible to assess whether claims at allowance in each application will overlap requiring a terminal disclaimer until there is an indication of allowable subject matter in at least one application, and for certainty, both applications. It is premature to file a terminal disclaimer at this time. Therefore, deferral of this resolution of this issue is respectfully is requested.

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Consideration of the above remarks, entry of this amendment and continued examination of the application on the merits respectfully are requested.

Respectfully submitted,

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